Analytical Methods Used in the Production and Fuel Quality Assessment of Biodiesel

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Abstract. *Biodiesel, an alternative diesel fuel derived from vegetable oil, animal fats, or waste vegetable oils, is obtained by reacting the oil or fat with an alcohol (transesterification) in the presence of a basic catalyst to give the corresponding mono–alkyl esters. Two major categories of methods besides other miscellaneous ones have been reported in the literature for assessing biodiesel fuel quality and/or monitoring the transesterification reaction by which biodiesel is produced. The two major categories comprise chromatographic and spectroscopic methods. This article considers the various methods in each category, including advantages and drawbacks, and offers suggestions on selection of appropriate methods.*

Keywords. *Biodiesel, Chromatographic methods, Fiber–optic probe, Fuel quality, Gas chromatography, Gel permeation chromatography, High–performance liquid chromatography, Mass spectrometry, Near–infrared spectroscopy, Nuclear magnetic resonance spectroscopy, Physical properties, Spectroscopic methods, Transesterification, Viscometry.*

Biodiesel has significant potential for use as an alternative fuel in compression–ignition (diesel) engines (Knothe et al., 1997; Dunn et al., 1997). It is technically competitive with conventional, petroleum–derived diesel fuel and requires no changes in the fuel distribution infrastructure. While some technical improvements regarding cold–flow properties, reduction of NO_x exhaust emissions, and oxidative stability remain, a major hurdle towards widespread commercialization is the high price of biodiesel. For this reason, in the United States the commercialization of biodiesel is targeted towards regulated fleets and mining and marine markets. In these markets, environmental and energy security concerns, which are subject to legislation, can override economic aspects.

Vegetable oils, such as soybean oil, rapeseed oil (canola oil), and in countries with more tropical climates, tropical oils (palm oil and coconut oil) are the major sources of biodiesel. However, in recent years, animal fats and especially recycled greases and used vegetable oils have found increasing attention as sources of biodiesel, the latter primarily as inexpensive feedstocks (Mittelbach and Tritthart, 1988). Regardless of the feedstock, transesterification reactions are carried out to produce biodiesel. Early on during the renewed interest in vegetable oils as alternative diesel fuels, it was observed that the resulting vegetable oil (or animal fat) esters did not exhibit the operational problems, such as engine deposits, coking of injector nozzles, etc., associated with neat oils (Bruwer et al., 1980).

The transesterification reaction of triacylglycerols in vegetable oils, usually with a monohydric alcohol such as methanol, in the presence of a basic catalyst such as NaOH or KOH, yields the mono–alkyl esters of the fatty acids, mainly comprising vegetable oils besides glycerol as side product (see figure 1). Methanol, being the least expensive alcohol, is the most commonly used alcohol for transesterification and accordingly yields methyl esters such as methyl soyate and rapeseed methyl ester. Recently, modifications of the transesterification reaction, such as those based on enzymatic catalysis, have been explored (Nelson et al., 1996).

During the transesterification process, intermediate glycerols, mono– and diacylglycerols, are formed, small amounts of which can remain in the final biodiesel (methyl ester) product. Besides these partial glycerols, unreacted triacylglycerols, unseparated glycerol, free fatty acids, residual alcohol, and catalyst can contaminate the final product. The contaminants can lead to severe operational problems when using biodiesel, such as engine deposits, filter clogging, or fuel deterioration. Therefore, in the United States an ASTM (American Society for Testing and Materials) standard is under development (Howell, 1997). In some European countries, such as Austria, the Czech Republic, France, Germany, and Italy, standards have been developed that limit the amount of contaminants in biodiesel fuel. In these standards, restrictions are placed on the individual contaminants by inclusion of items such as free and total glycerol for limiting glycerol and acylglycerols, flash point for limiting residual alcohol, acid value for limiting free fatty acids, and ash value for limiting residual catalyst. A more detailed discussion of the rationale for each quality parameter in biodiesel fuel standards is given in the literature (Mittelbach, 1994; Mittelbach, 1996). Another paper (Komers et al., 1998) briefly describes some methods used in the analysis of biodiesel, which include procedures for determining contaminants such as water and phosphorus that will not be dealt with here. The determination of biodiesel fuel quality is therefore an issue of great importance to the successful commercialization of this fuel. Continuously high fuel quality with no operational problems is a prerequisite for market acceptance of biodiesel. Accordingly, the analysis of biodiesel and the monitoring of the transesterification reaction have been the subject of numerous recent publications.

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Product names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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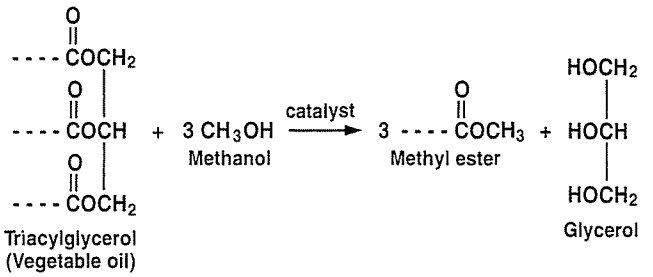

Figure 1. The transesterification reaction of triacylglycerols with methanol, yielding methyl esters and glycerol. The catalyst is usually a base, such as sodium or potassium hydroxide.

Generally, the major categories of analytical procedures for biodiesel comprise chromatographic and spectroscopic methods, although papers dealing with other methods, including physical property–based ones, have also appeared. The different categories and procedures will be evaluated here. As a rule, primarily research with direct reference to biodiesel analysis will be considered. Additional literature is available through the papers cited in the References.

General Aspects

The ideal analytical method for a product such as biodiesel would be able to reliably and inexpensively quantify all contaminants even at trace levels with experimental ease in a matter of, at most, seconds or even faster for on–line reaction monitoring. No current analytical method meets these extreme demands. Therefore, compromises are necessary when selecting methods for analyzing biodiesel or monitoring the transesterification reaction.

The categories mentioned above often overlap in organic analytical chemistry due to the advent of hyphenated techniques such as gas chromatography – mass spectrometry (GC–MS), gas chromatography – infrared spectrometry (GC–IR), and liquid chromatography – mass spectrometry (LC–MS). Few reports exist in the literature on the use of hyphenated techniques in biodiesel analysis. The main reasons are likely the higher equipment costs and the higher investment in technical skills of personnel needed to interpret the data. This is the case despite the fact that hyphenated techniques could aid in resolving ambiguities remaining after analysis by stand–alone chromatographic methods.

It is important to note that, in order to satisfy the requirements of biodiesel standards, the quantitation of individual compounds in biodiesel is not necessary but the quantitation of *classes of compounds* is. For example, for the determination of total glycerol, it does not matter which monoacylglycerol (for example, monolein or monostearin) the glycerol stems from. The same observation, of course, holds for di– and triacylglycerols. It does not even matter for the determination of total glycerol which type of acylglycerol (mono–, di– or tri–) the glycerol stems from. That acylglycerols are quantifiable as classes of compounds by GC is a result of the method.

Furthermore, virtually all methods used in the analysis of biodiesel are suitable (if necessary, with appropriate modifications) for all biodiesel feedstocks, even if the authors report their methods on one specific feedstock.

Chromatographic Methods

Both GC and HPLC analyses and combinations thereof have been reported for biodiesel. Gel permeation chromatography (GPC) as an analytical tool for analysis of transesterification products has also been reported. To date, most chromatographic analyses have been applied to methyl esters and not higher esters such as ethyl, *iso*–propyl, etc. It is likely that most methods discussed here would have to be modified to properly analyze the higher esters. For example, when conducting gas chromatographic analyses, changes in the temperature programs or other parameters may be necessary. The original work (Freedman et al., 1986) on GC analysis reported the investigation of methyl and butyl esters of soybean oil. Apparently, not all individual components could be separated there in the analysis of butyl soyate, but classes of compounds could be analyzed. HPLC analysis was applied to some ethyl, *iso*–propyl, 2–butyl, and *iso*–butyl esters of soybean oil and tallow (Foglia and Jones, 1997). If an analytical method has been applied to esters higher than methyl, it is noted here accordingly.

The first report on chromatographic analysis of the transesterification used thin layer chromatography with flame ionization detection (TLC / FID; Iatroscan instrument) (Freedman et al., 1984). In another report (Cvengros and Cvengrosová, 1994), TLC / FID was used to correlate bound glycerol content to acyl conversion determined by GC. It was found in this work that if conversion to methyl esters is > 96%, then the amount of bound glycerol is < 0.25 wt.–%. Although the TLC / FID method is easy to learn and use (Freedman et., 1984), it has been largely abandoned because of lower accuracy and material inconsistencies, as well as sensitivity to humidity (Freedman et al., 1984) and the relatively high cost of the instrument (Cvengros and Cvengrosová, 1994).

Gas Chromatography

Gas chromatography has to date been the most widely used method for the analysis of biodiesel due to its generally higher accuracy in quantifying minor components. Note, however, that accuracy of GC analyses can be influenced by factors such as baseline drift, overlapping signals, etc. It is not always clear that such factors are compensated for in such reports on biodiesel analysis. The first report on the use of capillary gas chromatography discussed the quantitation of esters as well as mono–, di–, and triacylglycerols (Freedman et al., 1986). The samples were treated with *N,O–bis*(trimethylsilyl)trifluoracetamide (BSTFA) to give the corresponding trimethylsilyl (TMS) derivatives of the hydroxy groups. This kind of derivatization has been carried out in subsequent research on GC quantitation of biodiesel. Derivatization to TMS derivatives is important because it improves the chromatographic properties of the hydroxylated materials, and in case of coupling to a mass spectrometer, facilitates interpretation of their mass spectra. While the original research (Freedman et al., 1986) used a short (1.8 m) fused silica (100% dimethylpolysiloxane) capillary column, other research typically used fused–silica capillary columns coated with a 0.1–μm film of (5%–phenyl)–methylpolysiloxane of 10 to 15 m length. The analysis of rapeseed ethyl esters was also carried out on a GC instrument equipped an FID and with a 1.8 m × 4 mm i.d. packed column (Cvengrosová et al., 1997).

Most reports on the use of GC for biodiesel analysis employ flame–ionization detectors (FID), although the use of mass spectrometric detectors (MSD) would eliminate any ambiguities about the nature of the eluting materials since mass spectra unique to individual compounds would be obtained. Two papers exist in the literature in which the use of mass spectrometric detection is described (Mittelbach, 1993; Mittelbach et al., 1996). It can be surmised that the additional cost of MSDs (and mass spectral interpretation) plays a role in deterring the commercial adoption of this detection method, although the benefits of mass spectrometry would likely more than compensate for the costs.

The majority of GC–related papers discuss the determination of a specific contaminant or class of contaminants in the methyl esters. The original report on biodiesel GC analysis (Freedman et al., 1986) quantified mono–, di–, and triacylglycerols in methyl soyate on a short 100% dimethylpolysiloxane column (1.8 m × 0.32 mm i.d.). Similar reports on the quantitation of acylglycerols exist (Mariani et al., 1991; Plank and Lorbeer, 1992). The main differences are in the specifications of the columns — both (5%–phenyl)–methylpolysiloxane, with differences in parameters such as column length — and the temperature programs, as well as standards.

Other papers discuss the individual or combined determination of other potential contaminants, such as free glycerol or methanol. A paper describing the use of a mass selective detector deals with the determination of glycerol (Mittelbach, 1993) and, in an extension thereof, a second paper describes the simultaneous quantitation of glycerol and methanol (Mittelbach et al., 1996). Other authors have also reported the determination of glycerol (Bondioli et al., 1992a) or methanol (Bondioli et al., 1992b). Using the same gas chromatographic equipment as in the previous determination of glycerol (Bondioli et al., 1992a) with only a modification of the oven temperature program, methanol could be determined. Ethanol was used as a standard for response factor determination. The flash point of biodiesel from palm oil and methanol content were correlated. Underivatized glycerol was detected with 1,4–butanediol as a standard on a short 2 m glass column (4 mm i.d.) loaded with Chromosorb 101 (Bondioli et al., 1992a), while the other method used derivatization and a 60 m × 0.25 mm i.d. column with 0.25–µm film of (5%–phenyl)–methylpolysiloxane and was reported to be more sensitive (Mittelbach, 1993). The temperature program varied (starting lower when determining methanol) (Mittelbach, 1993; Mittelbach et al., 1996), otherwise the column was the same.

Two papers (Mittelbach, 1993; Mittelbach et al., 1996) discuss the use of mass spectrometry as a detection method besides flame ionization. In the determination of free glycerol in biodiesel by GC–MS, selected ion monitoring (SIM) mode was used to track the ions *m/z* 116 and 117 of *bis*–O–trimethylsilyl–1,4–butanediol (from silylation of the 1,4–butanediol standard) and *m/z* 147 and 205 of *tris*–O–trimethylsilyl–1,2,3–propanetriol (from silylation of glycerol). The detection limit was also improved for rapeseed methyl ester (RME) when using MS in SIM mode (10^{-5} %) compared to the FID detector (10^{-4} %) (Mittelbach, 1993). In straightforward extension of this work, the simultaneous detection of methanol and glycerol by MS in SIM mode was reported (Mittelbach et al., 1996). For detection of (silylated) methanol (trimethylmethoxysilane), peaks at *m/z* 59 and 89 were monitored, as were peaks at *m/z* 75 and 103 of the additional (silylated) standard ethanol (trimethylethoxysilane). Mass spectrometry in SIM mode has the additional advantage that interfering signals can be avoided, and thus the use of shorter columns is possible (Mittelbach et al., 1996).

A further extension of the aforementioned research is the simultaneous determination of glycerol as well as mono–, di–, and triacylglycerols simultaneously by GC (Plank and Lorbeer, 1995). Here and in previous work (Plank and Lorbeer, 1992; Plank and Lorbeer, 1995) 10 m (5%–phenyl)–methylpolysiloxane columns with 0.1–µm film — 0.25 mm i.d. in Plank and Lorbeer (1992) and 0.32 mm i.d. in Plank and Lorbeer (1995) — were used. Major differences were the lower starting temperature of the temperature program (Plank and Lorbeer, 1995) and the addition of a standard (1,2,4–butanetriol) for the glycerol analysis. In this work (Plank and Lorbeer, 1992; Plank and Lorbeer, 1995), a cool on–column injector was used instead of the more common split/splitless injector.

Non–glyceridic materials that can be present in biodiesel also have been analyzed by GC. Thus, the determination of sterols and sterol esters in biodiesel (Plank and Lorbeer, 1993) has been reported. The stated reason is that the influence of these compounds, which remain in vegetable oils after processing (and thus in biodiesel after transesterification because they are soluble in methyl esters), on biodiesel fuel quality has not been determined (Plank and Lorbeer, 1993). Detection was carried out with a flame–ionization detector, as in other GC–related research, although in this case MS detection would appear especially desirable. The method for detection of sterols (Plank and Lorbeer, 1993) is virtually identical to the other GC method reported by the same authors (Plank and Lorbeer, 1992). The only differences are the use of sterol standards and a slight modification of the GC temperature program to spread the sterol peaks under condensation or even overlapping of the peaks of the other classes of compounds. Derivatization was carried out again with BSTFA (with 1% trimethylchlorosilane), and the column again was a fused–silica capillary column coated with a 0.1–µm film of (5%–phenyl)–methylpolysiloxane. The total concentration of sterols in rapeseed methyl ester was reported to be 0.339–0.500%, and sterol esters were 0.588–0.722%. In another paper on analysis of sterol content in rapeseed methyl ester (Plank and Lorbeer, 1994a), the same authors reported a sterol content of 0.70–0.81%. Other authors (Mariani et al., 1991) also pointed out the presence of sterols and sterol esters in biodiesel, but no analytical data were given.

High–Performance Liquid Chromatography

A general advantage of HPLC compared to GC is that time– and reagent–consuming derivatizations are not necessary, which reduces analyses times. Nevertheless, there are fewer reports of HPLC applied to biodiesel than GC analysis. The first report on the use of HPLC (Trathnigg and Mittelbach, 1990) described the use of an isocratic solvent system (chloroform with an ethanol content of 0.6%) on a cyano–modified silica column coupled to two gel permeation chromatography (GPC) columns with density detection. This system allowed for the detection of mono–, di–, and triacylglycerols as well as methyl esters as classes of

compounds. The system was useful for quantitating various degrees of conversion of the transesterification reaction.

HPLC with pulsed amperometric detection (the detection limit is usually 10 to 100 times lower than for amperometric detection, and the detection limit is 1 μg/g) was used to determine the amount of free glycerol in vegetable oil esters (Lozano et al., 1996). The major advantage of this detection method was its high sensitivity. The simultaneous detection of residual alcohol is also possible with this technique.

Reaction mixtures obtained from lipase–catalyzed transesterification were analyzed by HPLC using an evaporative light scattering detector (ELSD) (Foglia and Jones, 1997). This method is able to quantitate product esters, free fatty acids, and the various forms of acylglycerols. A solvent system consisting of hexane and methyl *tert.*–butyl ether (each with 0.4% acetic acid) with a gradient elution profile was used. It can be applied to esters higher than methyl, as discussed above.

In an extensive study (Holcapek et al., 1999), reversed–phase HPLC was used with different detection methods: UV detection at 205 nm, evaporative light scattering detection, and atmospheric pressure chemical ionization mass spectrometry (APCI–MS) in positive–ion mode. Two gradient solvent systems were used: one consisting of mixing methanol (A) with 5:4 2–propanol / hexane (B) from 100% A to 50:50 A:B — a non–aqueous reversed phase (NARP) solvent system — and the other consisting of mixing water (A), acetonitrile (B), and 5:4 2–propanol / hexane (C) in two linear gradient steps (30:70 A:B at 0 min, 100% B in 10 min, 50:50 B:C in 20 min, and last isocratic 50:50 B:C for 5 min). The first solvent system was developed for rapid quantitation of the transesterification of rapeseed oil with methanol by comparing the peak areas of methyl esters and triglycerols. The contents of individual acids (using normalized peak areas) were subject to error, and the results differed for the various detection methods. The sensitivity and linearity of each detection method varied with the individual triacylglycerols. APCI–MS and ELSD had decreased sensitivity with increasing number of double bonds in the fatty acid methyl esters, while UV will not quantify the saturates. APCI–MS was stated to be the most suitable detection method for the analysis of rapeseed oil and biodiesel.

Gel Permeation Chromatography

One report exists which describes the use of GPC (which is very similar to HPLC in instrumentation except for the nature of the column and the underlying separation principle, namely molecular weight of the analytes for GPC) for the analysis of transesterification products (Darnoko et al., 2000). Using a refractive index detector and tetrahydrofuran as mobile phase, mono–, di–, and triacylglycerols as well as the methyl esters and glycerol could be analyzed. The method was tailored for palm oil, and standards were selected accordingly. Reproducibility was good, with the standard deviation at different rates of conversion being 0.27–3.87%.

Liquid Chromatography with Gas Chromatography

The combination of liquid chromatography (LC) with gas chromatography (GC) has also been reported. The purpose of the combination of the two separation methods is to reduce the complexity of the gas chromatograms and to obtain more reliable peak assignments (Lechner et al., 1997).

In a study on analytical methods for determining biodiesel in mixtures with conventional diesel fuel, silica cartridge (Sep–Pak) chromatography with hexane / diethyl ether as solvents was employed to separate biodiesel from conventional diesel fuel (Bondioli et al., 1994).

A fully automated LC–GC instrument was employed in the determination of acylglycerols in vegetable oil methyl esters (Lechner et al., 1997). Hydroxy groups were acetylated, and then the methyl esters (sterols and esterified sterols elute with methyl esters) and acylglycerols were pre–separated by LC (variable wavelength detector). The solvent system for LC was hexane / methylene chloride / acetonitrile 79.97:20:0.05 and GC (flame–ionization detector) was performed on a 10 m (5%–phenyl)–methylpolysiloxane column. One LC–GC run required 52 minutes.

LC–GC was also applied to the analysis of sterols in biodiesel derived from rapeseed oil (Plank and Lorbeer, 1994a; Plank and Lorbeer, 1994b). On–line LC–GC was applied to the analysis of sterols from five different types of methyl esters (Plank and Lorbeer, 1994b). The vegetable oil methyl esters were those of rapeseed oil, soybean oil, sunflower oil, high–oleic sunflower oil, and used frying oil. The sterols were silylated prior to analysis with *N*–methyl–*N*–trimethylsilyltrifluoracetamide (MSTFA). No saponification and off–line pre–separation was required. The methyl esters were separated from the sterols by LC with a hexane / methylene chloride / acetonitrile 79.9:20:0.1 solvent system. Gas chromatography was again carried out with a 12 m (5%–phenyl)–methylpolysiloxane column and FID detection. Total concentrations of free sterols were 0.20–0.35 weight–% for the five samples, while sterol esters displayed a range of 0.15–0.73 weight–%. Soybean oil methyl ester was at the lower end (0.20% and 0.15%, respectively), while rapeseed oil methyl ester was the higher end (0.33% and 0.73%, respectively). In a comparison of two methods, saponification and isolation of the sterol fraction with subsequent GC analysis and LC–GC analysis of sterols in rapeseed oil methyl ester (Plank and Lorbeer, 1994b), despite the sophisticated instrumentation required, LC–GC was recommended because of additional information, short analysis time, and reproducibility. The total sterol content in rapeseed methyl ester was found to be 0.70–0.81 weight–%.

Spectroscopic Methods

Spectroscopic methods also have been reported for the analysis of biodiesel and/or monitoring of the transesterification reaction. These methods are ^{1}H nuclear as well as ^{13}C magnetic resonance (NMR) spectroscopy and near–infrared (NIR) spectroscopy.

Nuclear Magnetic Resonance

The first report on spectroscopic determination of the yield of a transesterification reaction utilized ^{1}H–NMR (Gelbard et al., 1995). Figure 2 depicts the ^{1}H–NMR spectrum of a progressing transesterification reaction. These authors used the protons of the methylene group adjacent to the ester moiety in triglycerols and the protons in the alcohol moiety of the product methyl esters to monitor the yield. A

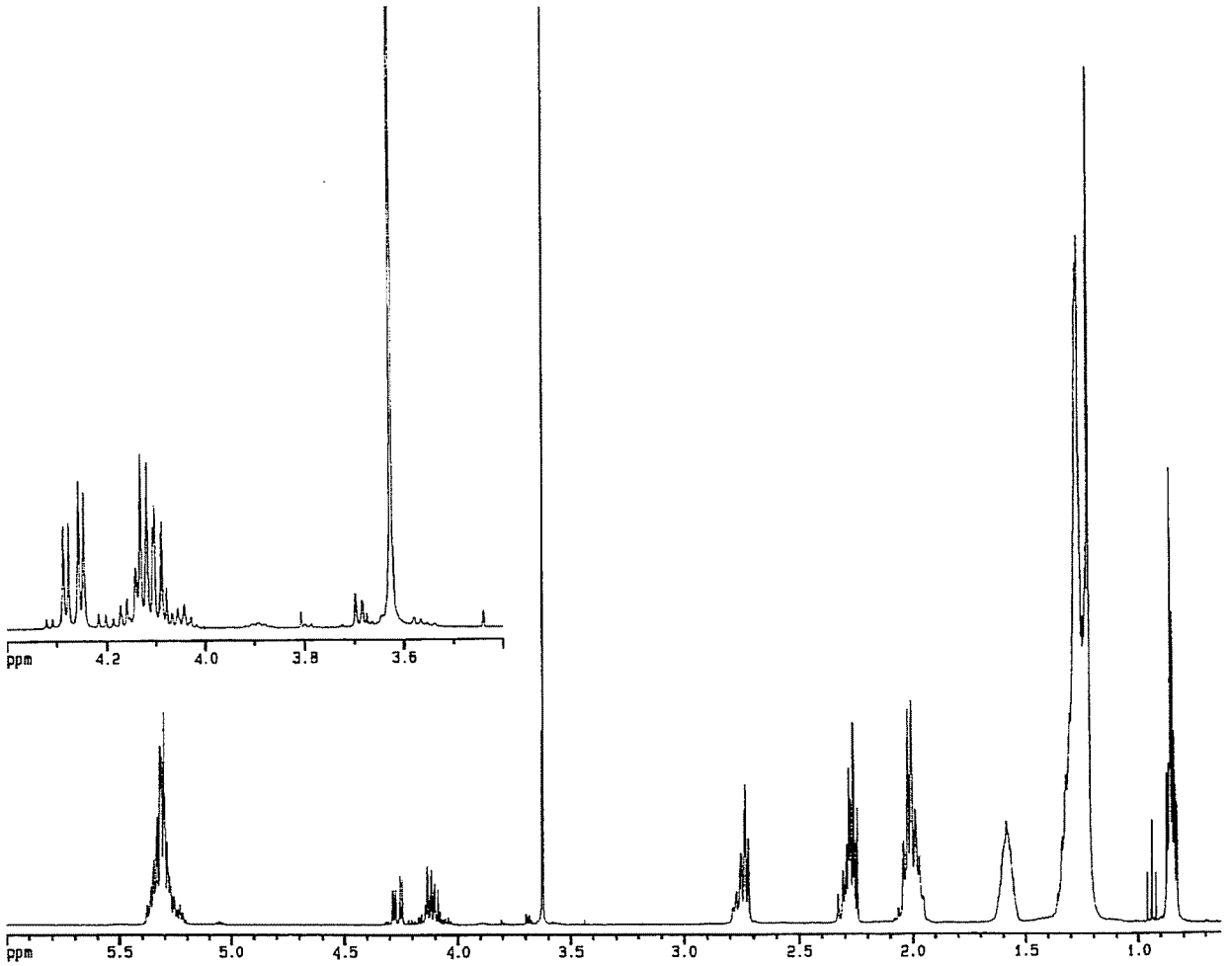

Figure 2. ^{1}H–NMR spectrum of a progressing transesterification reaction. The signals at 4.1–4.3 ppm are caused by the protons attached to the glycerol moiety of mono–, di–, or triacylglycerols. The strong singlet at 3.6 ppm indicates methyl ester ($-CO_2CH_3$) formation. The signals at 2.3 ppm result from the protons on the CH_2 groups adjacent to the methyl or glyceryl ester moieties ($-CH_2CO_2CH_3$ for methyl esters). These signals can be used for quantitation.

simple equation is given by the authors (their terminology is slightly modified here):

$$C = 100 \times \left(\frac{2A_{ME}}{3A_{-CH_2}} \right) \quad (1)$$

where

C = conversion of triacylglycerol feedstock (vegetable oil) to the corresponding methyl ester.

A_{ME} = integration value of the protons of the methyl esters (the strong singlet peak).

A_{-CH_2} = integration value of the methylene protons.

The factors 2 and 3 derive from the fact that the methylene carbon possesses two protons and the alcohol (methanol–derived) carbon has three attached protons.

Turnover and reaction kinetics of the transesterification of rapeseed oil with methanol were studied by ^{13}C–NMR (Dimmig et al., 1999) with benzene–d_6 as solvent. The signals at approximately 14.5 ppm of the terminal methyl groups unaffected by the transesterification were used as internal quantitation standard. The methyl signal of the product methyl esters registered at around 51 ppm, and the glyceridic carbons of the mono–, di–, and triacylglycerols registered at 62–71 ppm. Analysis of the latter peak range allowed the determination of transesterification kinetics, which showed that the formation of partial acylglycerols from the triglycerols is the slower, rate–determining step.

Near–Infrared Spectroscopy

More recently, NIR spectroscopy has been used to monitor the transesterification reaction (Knothe, 1999). The basis for quantitation of the turnover from triacylglycerol feedstock to methyl ester product are differences in the NIR spectra of these classes of compounds. At 6005 cm^{-1} and at 4425–4430 cm^{-1}, the methyl esters display peaks, while triacylglycerols only exhibit shoulders (see fig. 3). Ethyl esters could be distinguished in a similar fashion. (Knothe, 1999). Using quantitation software, it is possible to develop a method (using partial least squares regression) for quantifying the turnover of triacylglycerols to methyl esters. The absorption at 6005 cm^{-1} gave better results than the one at 4425 cm^{-1}. Note that the mid–range IR spectra of triacylglycerols and methyl esters of fatty acids are almost identical and offer no possibility for distinguishing. It appears that ethyl esters, and perhaps even higher ester, may be distinguished similarly by NIR from triacylglycerols, but no results have been reported yet.

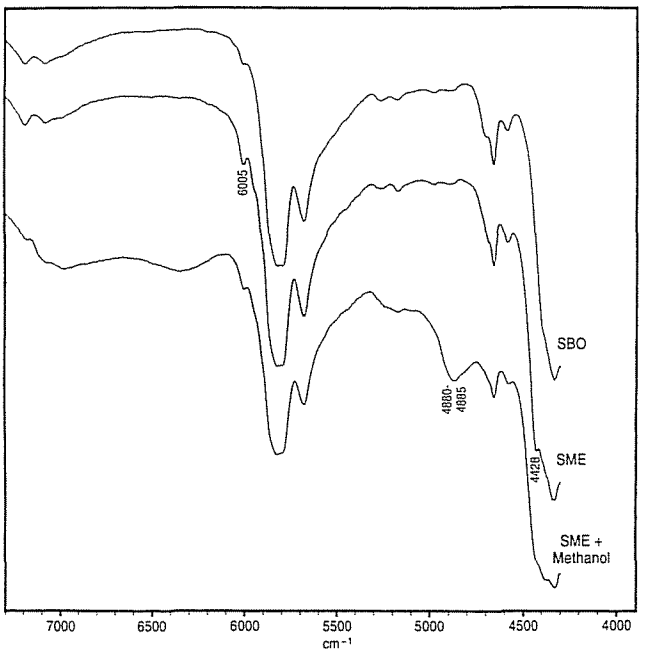

Figure 3. NIR spectra of soybean oil (SBO), methyl soyate (SME), and SME containing significant amounts of methanol. The inscribed wave numbers highlight the possibilities for distinguishing the spectra and thus quantifying the components.

NIR spectra were obtained with the aid of a fiber–optic probe coupled to the spectrometer, which renders their acquisition particularly easy and time–efficient.

Contaminants of biodiesel cannot be fully quantified by NIR at the low levels called for in biodiesel standards. The accuracy of the NIR method in distinguishing triacylglycerols and methyl esters is in the range of 1–1.5%, although in most cases better results are achieved. To circumvent this difficulty, an inductive method can be applied. The inductive method consists of verifying — by, say, gas chromatography — that a biodiesel sample meets prescribed biodiesel standards. The NIR spectrum of this sample would then be recorded. The NIR spectrum of the feedstock would also be recorded, as well as the spectra of intermediate samples at conversions of, say, 25%, 50%, and 75%. With these samples, a quantitative NIR evaluation method could be established. When another transesterification reaction is subsequently conducted, the NIR spectrum would indicate that the reaction (within the prescribed parameters such as time and temperature) has attained conversion to a product that (within experimental error of NIR) conforms to standards. It can be safely assumed that this result is correct, even if not all potential contaminants have been fully analyzed. Only if a significant deviation is indicated by NIR (beyond experimental error) would a detailed investigation by a more complex method such as GC be necessary. The NIR procedure is considerably less labor–intensive, faster, and easier to perform than GC.

While the first NIR paper used a model system to describe monitoring of transesterification and for developing quantitation methods, a second paper applied the method to a transesterification reaction in progress on a 6 L scale. Here, spectroscopic results were obtained not only by NIR but also by ^{1}H–NMR and NIR (Knothe, 2000). The results of both spectroscopic methods, which can be correlated by simple equations, were in good agreement. Two NMR approaches were used, one being the use of the methyl ester protons (peak at 3.6 ppm in figure 2) and the protons on the carbons next to the glyceryl moiety (: $-CH_2$; peaks at 2.3 ppm in figure 2) (Knothe, 2000), for which the equation for determining conversion (in %) is:

$$C_{ME} = 100 \times \frac{5 \times I_{ME}}{5 \times I_{ME} + 9 \times I_{TAG}} \qquad (2)$$

where I refers to integration values, and the subscripts ME and TAG refer to methyl esters and triacylglycerol. The second approach was the use of the methyl ester protons and the protons of the glyceryl moiety (peaks at 4.1–4.3 ppm in figure 2) in the triacylglycerols (Knothe, 2000).

In connection with the spectroscopic assessment of biodiesel fuel quality and monitoring of the transesterification reaction, a paper that discusses determining the amount of biodiesel in lubricating oil (Sadeghi–Jorabchi et al., 1994) is noteworthy. The investigated problem is significant because biodiesel can cause dilution of the lubricant, which can ultimately result in engine failure. The dilution was attributed to the higher boiling range of biodiesel (Sadeghi–Jorabchi et al., 1994; Siekmann et al., 1982) compared to conventional diesel fuel, whose more volatile components have less chance to dilute the lubricant. These authors used mid–range IR spectroscopy with a fiber–optic probe to determine the amount of biodiesel in lubricating oil. The range used was 1820–1680 cm^{-1}, which is typical for carbonyl absorption and which is not observed in conventional diesel fuel nor in the lubricating oil (note that this range is not suitable for distinguishing vegetable oils and their methyl esters because they have nearly identical carbonyl absorptions in the mid–IR range). Previous to this work, other authors had used IR spectroscopy (without the aid of a fiber–optic probe) in the range 1850–1700 cm^{-1} to analyze biodiesel in lubricating oil (Siekmann et al., 1982). The carbonyl absorption at 1750 cm^{-1} was not disturbed by the absorption of oxidation products at 1710 cm^{-1}.

Other Methods

Viscometry

The viscosity difference between component triacylglycerols of vegetable oils and their corresponding methyl esters resulting from transesterification is approximately one order of magnitude (Knothe et al., 1997; Dunn et al., 1997). For example, the viscosity of soybean oil is 32.6 mm^2/s (38°C) and that of methyl soyate is 4.41 mm^2/s (40°C) (Knothe et al., 1997, and references therein). The high viscosity of the vegetable oils was the cause of severe operational problems, such as engine deposits (Bruwer et al., 1980; Knothe et al., 1997; Dunn et al., 1997). This is a major reason why neat vegetable oils largely have been abandoned as alternative diesel fuels in favor of mono–alkyl esters such as methyl esters.

The viscosity difference forms the basis of an analytical method, viscometry, applied to determining the conversion of vegetable oil to methyl ester (De Filippis et al., 1995). Viscosities determined at 20°C and 37.8°C were in good agreement with GC analyses conducted for verification

purposes. The viscometric method, especially results obtained at 20°C, is reported to be suitable for process–control purposes due to its rapidity (De Filippis et al., 1995). Similar results were obtained from density measurements (De Filippis et al., 1995).

Titration for Determining Free Fatty Acids

Titration methods for determining the neutralization number (NN) of biodiesel were described (Komers et al., 1997). Two methods for determining strong acids and free fatty acids in one measurement were developed. One method, of particular interest, used potentiometry, while the other used two acid–base indicators (neutral red, phenolphthalein). The potentiometric method is more reliable, and even with the use of two indicators, the NN values derived from the titration method are 10–20 rel.–% greater than the real acidity of the sample.

Enzymatic Methods

An enzymatic method for analyzing glycerol in biodiesel was described to test for completeness of the transesterification reaction (Bailer and de Hueber, 1991). Solid–phase extraction of the reaction mixture with subsequent enzymatic analysis was applied. This method was originally intended as a simple method for glycerol determination, but reproducibility and complexity concerns exist (Bondioli et al., 1992a; Lozano et al., 1996).

Biodiesel Blends

Blends of biodiesel with conventional diesel fuel represent a common utilization of biodiesel. In the United States, "B20" (a blend of 20% biodiesel with 80% conventional diesel fuel) is recognized as an alternative diesel fuel under criteria of the Energy Policy Act (EPACT). In France, biodiesel is utilized as a lower–level blend with conventional diesel fuel. In this connection, a problem that has found little attention to date in the literature is determining or verifying the blend level of biodiesel in conventional diesel fuel in such blends. NIR spectroscopy using the same peaks for quantitation as those for monitoring transesterification and fuel quality appears to be suitable for such purposes (Knothe, manuscript submitted for publication). Mid–IR spectroscopy as used for the determination of biodiesel in lubricating oil (Sadeghi–Jorabchi et al., 1994) should also be suitable. The use of the NIR range, however, would permit using a spectrometer without any changes in instrument settings for reaction and fuel quality monitoring as well as for determining blend levels. Generally, it appears that spectroscopic methods may be more suitable to address the problem of determining / verifying blend levels. Especially GC would appear to yield very complex chromatograms due to the numerous components of conventional diesel fuel. In HPLC, as done previously, the classes of compounds may elute and be analyzable, thus reducing complexity. Methods based on physical properties may also be suitable for determining biodiesel blend levels.

Summary and Outlook

Several analytical methods have been investigated for fuel quality assessment and production monitoring of biodiesel. The most intensively studied method is GC, while HPLC, NMR, and NIR have also been studied. GC is also the method used for verification that biodiesel meets prescribed standards due to its ability to detect low–level contaminants, although improvements to this method are possible. Physical properties–based methods have been explored less, and it appears that this may be an area for further study. However, no method can simultaneously satisfy all criteria of simultaneously determining all trace contaminants with minimal investment of time, cost, and labor. The following approach therefore appears to be a reasonable compromise. A fast and easy–to–use method that may be adaptable to production monitoring, such as NIR (or viscometry), can be used for routine analyses. For example, if measurements by NIR (or viscometry) at several turnover ratios indicate that the transesterification reaction is progressing as desired and that the NIR spectrum (viscosity) of the biodiesel product agrees with that of one known to meet biodiesel standards, then further, more complex, analyses would be unnecessary. Only if NIR (or viscometry) analyses indicate that there is a potential problem with the product would more complex and time–consuming analyses, for example by GC, be warranted to determine the exact cause of the problem.

Determining the blend levels of biodiesel in blends with conventional diesel fuel is also an area that can be expanded.

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